(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 November 2001 (15.11.2001)

PCT

(10) International Publication Number
WO 01/85146 A1

(51) International Patent Classification[7]: A61K 31/00, 31/136, 31/4706, A61P 11/00

(21) International Application Number: PCT/SE01/01014

(22) International Filing Date: 8 May 2001 (08.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0011358.9 12 May 2000 (12.05.2000) GB

(71) Applicant *(for all designated States except US)*: **ASTRAZENECA AB** [SE/SE]; S-151 85 Södertälje (SE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **THONG, Kam-Wah** [GB/GB]; AstraZeneca R & D Charnwood, Bakewell Road, Loughborough, Leics. LE11 5RH (GB).

(74) Agent: **ASTRAZENECA AB**; Global Intellectual Property, S-151 85 Södertälje (SE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/85146 A1

(54) Title: PHARMACEUTICAL COMPOUNDS FOR TREATING COPD

(57) Abstract: Use of an MPO inhibitor for the treatment of COPD.

Pharmaceutical compounds for treating COPD.

The present invention relates to the use of certain pharmaceutical compounds for the treatment of COPD.

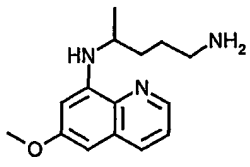
COPD is a major cause of morbidity and mortality. A key etiological factor is smoking. It is apparent that smokers have elevated levels of MPO (Dash *et al.*, Blood., 1991, 72, 1619; Bridges *et al.*, Eur. J. Respir. Dis., 1985, 67, 84). Furthermore, there is circumstantial evidence to link MPO levels with the severity of lung disease in human subjects (Hill *et al.*, Am. J. Respir. Crit. Care., 1999, 160, 893; Keatings & Barnes., Am. J. Respir. Crit. Care., 1997, 155, 449; Regelmann *et al.*, Pediatric. Pulmonol., 1995, 19, 1; Linden *et al.*, Am Rev. respir. Dis., 1993, 148, 1226). Myeloperoxidase is a heme protein that plays a vital role in the generation of toxic hypochlorus acid and free radicals, which may be involved in cellular damage and inflammation (Kettle & Winterbourn., Curr. Opin. Hematol., 2000, 7, 53). This protein has been implicated in a variety of different conditions (Klebanoff., Proc. Assoc. Am. Physicians., 1999, 111, 383). Compounds having activity as inhibitors of MPO are known in the art (Kettle & Winterbourn., Biochem. Pharmacol., 1991, 41, 10; Bozeman *et al.*, Biochem. Pharmacol., 1992, 44, 553). For example, the compound dapsone, which is known to be an inhibitor of MPO has been linked to the treatment of various conditions, including a general reference to inflammatory diseases such as asthma (Berlow *et al.*, J. Allergy Clin. Immunol., 1991, 87, 710). Interestingly, there is no specific mention of any synthetically derived chemical inhibitors of MPO being use for the treatment of COPD.

Current drugs used for treating COPD are not all fully effective. The need for novel and better drugs is essential to cope with the rising incidence of COPD (Peleman *et al.*, Curr. Opin. Cardiovas. Pulmonary. Renal. Invest. Drugs., 1999, 1, 491). It has now surprisingly been found that compounds having activity as inhibitors of MPO are expected to be of potential use in the treatment of COPD.

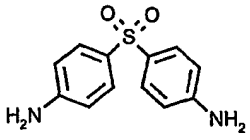
In a first aspect the invention therefore provides the use of an MPO inhibitor for the treatment of COPD. It will be understood that the MPO inhibitors of the invention can be used therapeutically or as prophylactics.

Particularly suitable compounds include MPO inhibitors known in the art.

Preferred compounds include those listed below:

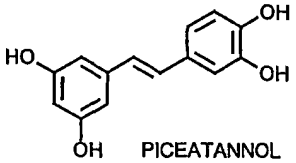


PRIMAQUINE

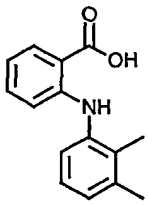


DAPSONE

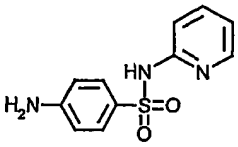
AMINOPYRINE



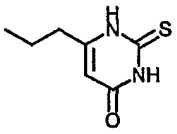
PICEATANNOL



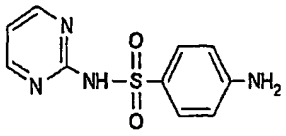
MEFENAMIC ACID



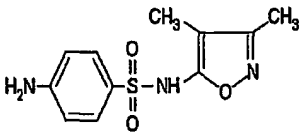
SULFAPYRIDINE



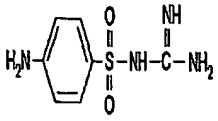
PROPYLTHIOURACIL



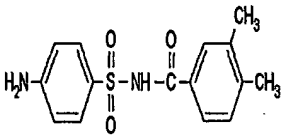
SULFADIAZINE



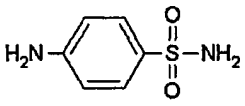
SULFISOXAZOLE



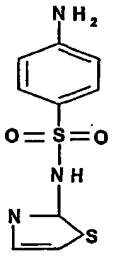
SULFAGUANIDINE



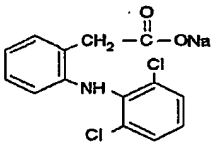
SULFANITRAN



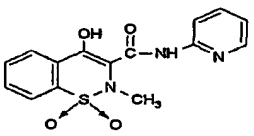
SULFANILAMIDE



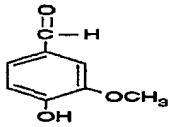
N-1(2 THIAZOLYL)SULFANILAMIDE



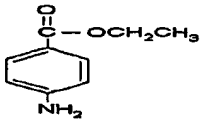
DICLOFENAC



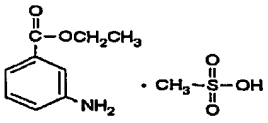
PIROXICAM



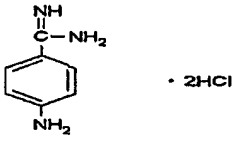
VANILLIN



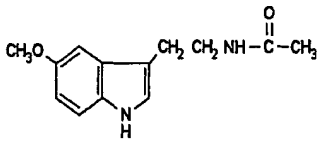
ETHYL AMINOBENZOATE



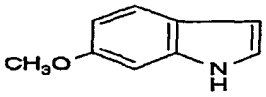
3 AMINO ACID ETHYLESTER



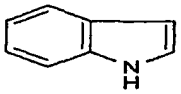
p-AMINOBENZAMIDINE



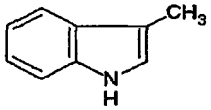
MELATONIN



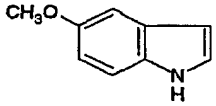
6 METHOXYINDOLE



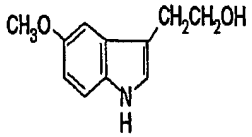
INDOLE



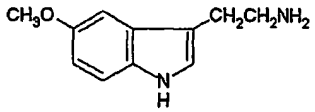
3-METHYLINDOLE



5-METHOXYINDOLE



5 METHOXYTRYPTOPHOL



5 METHOXYTRYPTAMINE

Additional preferred compounds include the following:

ISONIAZID

NITECAPONE

5-AMINOSALICYLIC ACID

PHENYLHYDRAZINE

D-PENICILLAMINE

TIOPRONIN

RESORCINOL

QUERCETIN

RUTIN

QUINACRINE

BAKUCHIOL

The above compounds can be used both as free bases and pharmaceutically acceptable salts. Suitable salts include all known pharmaceutically acceptable salts such as acid addition salts such as hydrochloride and malate salts.

Preferred compounds include primaquine, sulfanilamide, dapsone and sulfapyridine, in particular dapsone.

The invention also provides a method of treating or preventing COPD, which comprises administering to a patient an MPO inhibitor or a pharmaceutically acceptable salt thereof in particular by administering primaquine, dapsone, aminopyrine, piceatannol, mefenamic acid, sulfapyridine, sulfanilamide, propylthiouracil, sulfadiazine, sulfisoxazole, sulfaguanidine, sulfanitran, sulfanilamide, N-1(2-thiazolyl)sulfanilamide, diclofenac, piroxicam, vanillin, ethyl aminobenzoate, 3-aminoacid ethylester, p-aminobenzamide, melatonin, 6-methoxyindole, indole, 3-methylindole, 5-methoxyindiole, 5-methoxytryptophol, 5-methoxytryptamine and pharmaceutically acceptable salts thereof.

In a further aspect the invention provides an MPO inhibitor, in particular a compound named above, in the manufacture of a medicament for use in the prevention or treatment of COPD.

Suitable daily dose ranges are from about 0.1 mg/kg to about 100 mg/kg. Unit doses may be administered conventionally once or more than once a day, for example, 2, 3, or 4 times a day, more usually 1 or 2 times a day. A typical dosing regime for dapsone or propylthiouracil would be oral once or twice a day at 100 mg or 300mg, respectively.

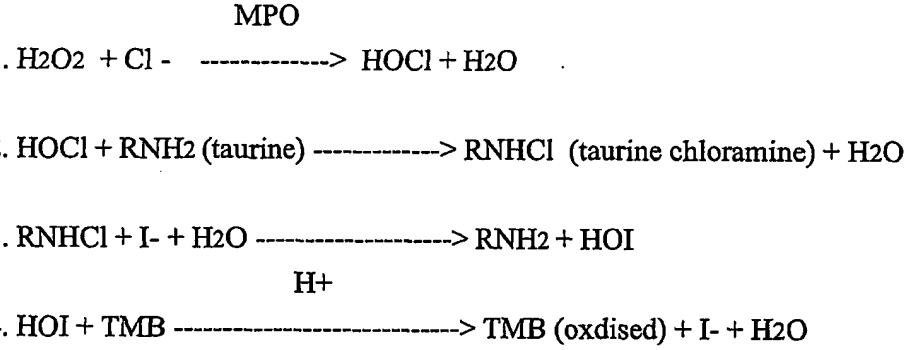
The pharmaceutical composition comprising the MPO inhibitor of the invention may conveniently be in the form of tablets, pills, capsules, syrups, powders or granules for oral

administration; sterile parental or subcutaneous solutions, suspensions for parental administration of suppositories for rectal administration, all of which are well known in the art.

The following examples illustrate the invention.

Example 1

Here we describe an *in vitro* MPO assay that was developed to assess inhibition of enzyme activity. Essentially the MPO assay was designed to measure the production of hypochlorus acid (HOCl), which is the key physiological product generated by the enzyme *in vivo*. An outline of the assay reactions is given:



The reaction mixtures in 20mM phosphate buffer pH6.5 contained 2.5nM MPO (purified human enzyme from Planta), 100uM H_2O_2, 140mM NaCl, 10mM taurine, 20uM tyrosine and compound solvent, DMSO, at 1%. Compounds were preincubated with the MPO enzyme in buffer for about 15min prior to start of reaction with H_2O_2. The whole reaction was carried out at room temperatutre for 10min in a 96-well plate. The reaction was terminated by a stop/developing reagent, which consist in their final concentration of Glacial acetic acid (400mM), KI (100uM) and TMB in dimethylformamide (10mM). All test concentrations were done in duplicated with at least two separate determinations n=2, unless otherwise stated. The inhibitory concentration for a compound is presentated as pIC50, which is –log IC50.

Various compounds have been tested against the human MPO. It can be seen that dapsone is the most potent inhibitor of the sulfones/sulfonamides tested. Indoles and other compounds are also effective in blocking the production of HOCl by human MPO. All data obtained for the sulfones/sulfonamides, indoles and miscellaneous are presented in Table 1, 2 and 3, respectively.

Table 1: Inhibtion of human MPO-HOCl production by sulfones/sulfonamides

Compound	pIC50
Dapsone	6.2
N-1(2 thiazolyl)-sulfanilamide	6.0
Sulfanilamide	6.0
Sulfapyridine	5.7
Sulfaguanidine	5.5
Sulfisoxazole	5.2
Sulfadiazine	5.2
Sulfanitran	5.1

Table 2: Inhibition of human MPO-HOCl production by indoles

Compound	pIC50
5-Methoxytryptophol	6.3
5-Methoxytryptamine	6.2
Melatonin	6.1
3-Methylindole	5.9
6-Methoxyindole	5.8
Indole	5.7
5-Methoxyindole	5.6

Table 3: Inhibition of human MPO-HOCl production

Compound	pIC50
Ethyl aminobenzoate	6.2
3-Aminobenzoic acid ethylester	6.2
p-Aminobenzamidine	5.6
Piroxicam	5.6 (n=1)
Diclofenac	5.4
Vanillin	5.1

Example 2

Here we describe the use of a functional human neutrophil assay to determine the effects of MPO inhibitors on the production of HOCl. This assay detects the production of HOCl from stimulated (e.g. PMA, LPS, fMLP, zymozan) human neutrophils. Human neutrophils were purified from fresh heparinised blood by density centrifugation on Polymorphprep (Nycomed). These neutrophils were used immediately after purification. A standard reaction mixture contained the following: 2 X 106 neutrophils, 140mM NaCl, 5mM taurine, 0.5mM MgCl2, 1mM CaCl2 and 1mg/ml glucose. Test compounds were made up in DMSO and added to cells, with a final DMSO concentration of 0.5%. Test compounds were given 15min preincubation at 37C with neutrophils prior to the addition of the PMA stimulant (1μg/ml). The assay was then allowed to progress for another 30min at 37C. At the end of the incubation, supernatants were collected by centrifugation and assayed for HOCl by using the stop/development reagent as above. All compounds were tested in duplicate with at least two separate determinations n=2 from two different donors.

The data for some of these inhibitors are shown in Table 4.

Table 4: Inhibition of HOCl production by stimulated human neutrophils

HOCl production by neutrophils	**pIC50**
Primaquine	4.9
Sufanilamide	4.8
Dapsone	4.7
Sulfapyridine	4.5

We have also shown that under the assay conditions and concentrations of inhibitors used, human neutrophils were not affected by cytotoxicity, as assessed by the release of lactate dehydrogenase from damaged neutrophils. Lactate dehydrogenase activity was measured

as described by Boehringer Mannheim GmbH, Sandhofer Strabe 116, D-68305 Mannheim, Germany (Cytotoxicity Detection Kit-LDH- Cat No: 1 644 793).

Example 3

There are several animal models of COPD, which can be employed for the testing of MPO inhibitors. These models have been referred in the reviews of Snider (Chest., 1992, 101, 74S) and Shapiro (Am. J. Respir. Cell Mol. Biol., 2000, 22, 4). In our study, we prefer the LPS- and/or smoking-induced lung injury rodent model. Mice or rats can be be dosed (by any of the following routes: ip, po, iv, sc or aerosol) with MPO inhibitors prior to LPS and/or smoking challenge. After an appropriate set interval, the animals are sacrificed and assessed for lung injury (similar to the work reported by Faffe *et al.*, Eur.Respir. J., 2000, 15, 85; Suntres & Shek., Biochem. Pharmacol., 2000, 59, 1155; Vanhelden *et al.*, Exp. Lung. Res., 1997, 23, 297). MPO activity of the lung lavage fluids (BAL), lung tissues, neutrophils and whole blood are then measured. Blood samples can be analysed for inflammatory cells and cytokines (e.g. TNFα). Histology and biochemical markers (e.g. chlorinated protein, lactate dehydrogenase, alkaline phosphatase) for lung cellular damage can be assessed. The efficacies of the MPO inhibitors are measured against their abilities to reduce/prevent lung injury. It is expected these MPO inhibitors will be therapeutically or prohylactically effective in these models.

CLAIMS

1. Use of an MPO inhibitor for the treatment of COPD.

2. Use according to claim 1 where the compound having MPO inhibitory activity is primaquine, dapsone, aminopyrine, piceatannol, mefenamic acid, sulfapyridine, sulfanilamide, propylthiouracil, sulfadiazine, sulfisoxazole, sulfaguanidine, sulfanitran, sulfanilamide, N-1(2-thiazolyl)sulfanilamide, diclofenac, piroxicam, vanillin, ethyl aminobenzoate, 3-aminoacid ethylester, p-aminobenzamide, melatonin, 6-methoxyindole, indole, 3-methylindole, 5-methoxyindiole, 5-methoxytryptophol, 5-methoxytryptamine and pharmaceutically acceptable salts thereof.

3. A method of treating or preventing COPD in a mammal which comprises administering a compound having MPO inhibiting activity or a pharmaceutically acceptable salt thereof.

4. A method according to claim 3 in which the MPO inhibitor is selected from primaquine, dapsone, aminopyrine, piceatannol, mefenamic acid, sulfapyridine, sulfanilamide, propylthiouracil, sulfadiazine, sulfisoxazole, sulfaguanidine, sulfanitran, sulfanilamide, N-1(2-thiazolyl)sulfanilamide, diclofenac, piroxicam, vanillin, ethyl aminobenzoate, 3-aminoacid ethylester, p-aminobenzamide, melatonin, 6-methoxyindole, indole, 3-methylindole, 5-methoxyindiole, 5-methoxytryptophol, 5-methoxytryptamine and pharmaceutically acceptable salts thereof.

5. A pharmaceutical composition for treating or preventing COPD which contains an MPO inhibitor or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable carrier or excipient.

6. A composition according to claim 6 in which the MPO inhibitor is selected from primaquine, dapsone, aminopyrine, piceatannol, mefenamic acid, sulfapyridine,

sulfanilamide, propylthiouracil, sulfadiazine, sulfisoxazole, sulfaguanidine, sulfanitran, sulfanilamide, N-1(2-thiazolyl)sulfanilamide, diclofenac, piroxicam, vanillin, ethyl aminobenzoate, 3-aminoacid ethylester, p-aminobenzamide, melatonin, 6-methoxyindole, indole, 3-methylindole, 5-methoxyindiole, 5-methoxytryptophol, 5-methoxytryptamine and pharmaceutically acceptable salts thereof.

7. Use of an MPO inhibitor in the manufacture of a medicament for use in the prevention or treatment of COPD.

8. Use according to claim 7 in which the MPO inhibitor is selected from primaquine, dapsone, aminopyrine, piceatannol, mefenamic acid, sulfapyridine, sulfanilamide, propylthiouracil, sulfadiazine, sulfisoxazole, sulfaguanidine, sulfanitran, sulfanilamide, N-1(2-thiazolyl)sulfanilamide, diclofenac, piroxicam, vanillin, ethyl aminobenzoate, 3-aminoacid ethylester, p-aminobenzamide, melatonin, 6-methoxyindole, indole, 3-methylindole, 5-methoxyindiole, 5-methoxytryptophol, 5-methoxytryptamine and pharmaceutically acceptable salts thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 01/01014

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 31/00, A61K 31/136, A61K 31/4706, A61P 11/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CHEM.ABS.DATA. EPO-INTERNAL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Am Rev Respir Dis, Volume 131, 1985, W. J. Martin II et al, "Reduction of Neutrophil-mediated Injury to Pulmonary Endothelial Cells by Dapsone 1-3" page 544 - page 547	1-8
A	WO 0051598 A1 (SMITHKLINE BEECHAM CORPORATION), 8 Sept 2000 (08.09.00)	1-8
A	Biochemical Pharmacology, Volume 41, No 10, 1991, Anthony J. Kettle et al, "Mechanism of inhibition of myeloperoxidase by anti-inflammatory drugs" page 1485 - page 1492	1-8

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 Sept 2001	14 -09- 2001
Name and mailing address of the ISA Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Eva Johansson/EÖ Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 01/01014

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Eur Respir J, Volume 15, 2000, S.W. Crooks et al, "Bronchial inflammation in acute bacterial exacerbations of chronic bronchitis: the role of leukotriene B4" page 274 - page 280 -- --------	1-8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE01/01014

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-4
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Claims 1-4 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT
Information on patent family members

02/08/01

International application No.
PCT/SE 01/01014

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0051598 A1	08/09/00	AU 3386900 A	21/09/00